

PROJECT NUMBER: 6908
PROJECT TITLE: Smoke Condensate Studies
PROJECT LEADER: A. H. Warfield
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I. TSNA PRECURSORS

- A. Objective: To determine the precursors of MS TSNA.
- B. Results: Nicotine bitartrate (NBT), a potential candidate for microencapsulation and subsequent use as a model of unextracted nicotine, was synthesized in crystalline form by J. Paine. In order to prepare the crystals for microencapsulation, it was necessary to reduce the particle size to 90-150 μ . X-ray microscopy (K. Sanders) revealed that the NBT crystals were thin rectangular platelets 4-40 μ X <1 μ thick, which were agglomerated into larger particles. These particles would not pass through a 150 μ screen. Grinding in a mortar and pestle did not appreciably reduce the particle size.

Ascorbic acid (ASC) was applied to an RL made from a mixture of burley (Bu) and oriental (Or) CEL's on Bu base web (BW), and cigarettes prepared from this RL were smoked for TSNA determination. Blending Or and Bu CEL's produces a significant reduction in MS TSNA relative to that obtained from the same amount of BuCEL on BuBW, as shown earlier. The TSNA reduction was further increased by addition of ASC. Blending ASC into the mixed CEL's before addition to the BW was not as effective as overspraying the ASC onto the prepared RL. Previous studies indicate that ASC acts largely by increasing decomposition of endogenous TSNA. Therefore, these results suggest that OrCEL acts largely on the pyrosynthetic mechanism of TSNA formation.

- D. Plans: Attempt to grind the NBT in a mill capable of operating at liquid nitrogen temperatures. When correctly sized particles are obtained, submit samples for microencapsulation, and add to appropriate fillers. Determine the effect on MS TSNA deliveries. Evaluate results of FTIR-EGA investigation of the effect of added OrCEL on nitrogen-containing compounds. Also, plans are being made to study OrCEL fractions in order to identify the active component(s).

E. References:

Haut, S. A. Notebook 8595, pp. 154-155.
Morgan, W. R. Notebook 8579, pp. 67-68.
Sanders, K. Notebook No. 8612, pp. 166, 178.

II. TSNA DECOMPOSITION STUDIES

- A. Objective: To explore the thermal stability of TSNA and investigate methods to enhance thermally induced decompositions of these compounds.

- B. Results: Slow heating experiments designed to investigate the role of antioxidants in decomposition of TSNA were completed. NNN and mixtures of NNN with excess ascorbyl palmitate (AP) and propyl dihydroxydihydrocinnamate (propyl DHHC) were placed in a GC oven in a device which was connected to a thermal energy analyzer (TEA) via a pyrolysis oven. The GC oven was heated at 5°C/min. to 400°C with the pyrolysis oven off, and the temperature of NO evolution measured. Either antioxidant caused a decrease in evolution temperature from ~210°C to ~125°C, with no other peaks appearing, indicating that both compounds enhance decomposition of NNN. With the pyrolysis oven on, NO evolution due to distillation during heating can also be observed. Under these conditions, preliminary results suggest that there are differences in the effects caused by the two antioxidants.
- C. Plans: After identification of an OrCEL fraction with TSNA-reducing activity, further decomposition studies will be performed.
- D. References:
 Tickle, M. Notebook 8716, pp. 20-21.
 Morgan, W. R. Notebook 8579, pp. 67-68.

III. CROSSED SOLUBLES/BASE WEB STUDY (CHEMISTRY)

- A. Objective: To investigate the smoke chemistry of model cigarettes made from all possible combinations of solubles from bright, burley and oriental tobaccos on base webs from the three tobaccos.
- B. Results: Several separation methods were applied to BuCEL in order to obtain data on the relationship of Salmonella/microsome (S/M) activity to precursors present in specific fractions. The insolubles fraction was obtained simply by centrifuging BuCEL and suspending the insolubles in water for application to BrBW. The soluble fraction (BuSl) was subjected to ultrafiltration (1000MW cutoff) at 5 different concentrations, and the fractions either passing through or retained were stored for future application to BrBW. BuSl fractions obtained by butanol-water liquid-liquid extraction were prepared for application to BrBW as well as later fractionation by reversed phase HPLC.

An experiment in which $\text{Ca}(\text{OAc})_2$ was added to BuCEL/BrBW has been completed and preliminary conclusions have been drawn. Addition of Ca^{++} to BrBW alone or BuCEL/BrBW does not affect puff count if the total Ca^{++} is not higher than ~7 and 5.5%, respectively. TPM delivery of BrBW or BuCEL/BrBW is decreased almost linearly by adding $\text{Ca}(\text{OAc})_2$.

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- C. Plans: Apply fractions prepared to BrBW, prepare cigarettes, and smoke for S/M assay. R. Izac will apply C-18 HPLC for separation of the butanol-water fractions. Filler will be prepared containing varying ratios of BuCEL and $\text{Ca}(\text{OAc})_2$ on BrBW for determination of smoking characteristics and activity data.

D. Reference:

Magin, D. Notebook 8660, p. 51.

IV. OPTIMIZATION OF A LOW ACTIVITY MODEL: MS NITRIC OXIDE CONTENT

- A. Objective: To develop methods of reducing MS NO delivery for a low activity model.
- B. Results: Filler from a BCR reference blend was oversprayed with 3% ascorbyl palmitate to determine whether addition of this antioxidant might lower NO delivery. Analysis of the corresponding smoke by the peroxide/nitrate ion selective electrode procedure developed last month showed that the treatment yields a marginal 11% reduction in NO delivery compared to the untreated control.
- C. Plans: Spraying of squalene and neophytadiene as possible NO scrubbers on filler will be evaluated.
- D. Reference:
- Levins, R. J. Notebook 8672, p. 70.

V. SUPPORT FUNCTION: CONDENSATE PREPARATION

- A. Objective: To fabricate cigarettes, perform smokings, and prepare condensate as needed for biological and chemical analysis.
- B. Results: Thirteen samples prepared as part of the Crossed Solubles Base Web Study were sprayed on the specified fillers, and cigarettes made and smoked for S/M testing, along with the appropriate controls. In addition, 30 2R1 cigarettes were smoked and the smoke processed for EGF assay.
- C. Reference:
- Hellams, R. D. Notebook 8613, p. 111.

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